

Plausible microbial metabolisms on Mars

Sophie L Nixon, Claire R Cousins and Charles S Cockell explore the possible ways in which any martian life could get energy.

The surface of Mars is currently inimical to life, being both oxidizing and exposed to high levels of ultraviolet and ionizing radiation, and has been so for a significant part of its history. Oxygen exists in the martian atmosphere at trace levels only, meaning that the planet is anoxic. Anaerobic redox-driven metabolisms, which use thermodynamically favourable reduction–oxidation reactions to generate energy, operating within the deep- and near-subsurface, are therefore the most plausible energy pathways for life on Mars, past and present. Some of these redox reactions can operate independently of photosynthesis, meaning they can persist deep underground, while some may have once used geochemical disequilibria within near-surface hydrothermal systems. Here, we provide an overview of both the availability of geologically derived redox couples on Mars, and the known (and unknown) microbial metabolisms that could feasibly exploit them.

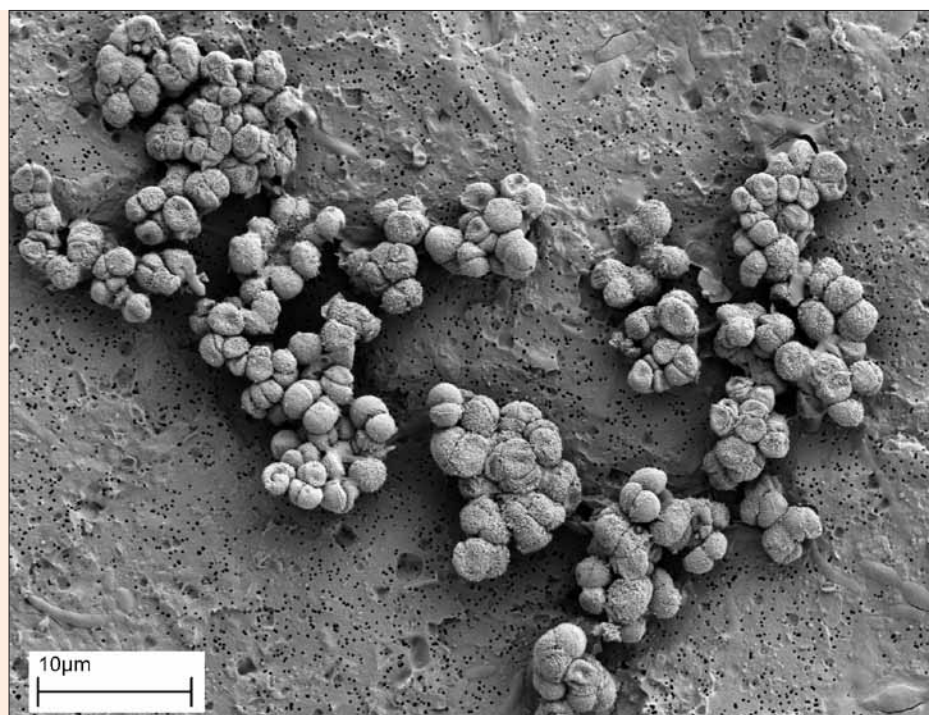
The martian environment

Hydrated mineralogical assemblages identified at the martian surface reveal a diversity of palaeoenvironments, many of which may have been habitable, based on our current understanding of the limits of terrestrial life. A broad global model of pH-neutral to increasingly acidic aqueous conditions over time (Bibring *et al.* 2006) implies decreasing near-surface habitability since the Noachian, with the present-day surface considered hostile to life. While the formation of these palaeoenvironments is open to interpretation, the hydrated mineral assemblages themselves reveal what chemical compounds and species were available for microbial metabolism. Iron (Fe) and sulphur (S) species in particular are globally widespread, with both S and Fe significantly more enriched within martian rocks and soils than on Earth (King and McLennan 2010, McSween *et al.* 2009). This enrichment is potentially explained by sustained Noachian–Hesperian volcanism releasing SO₂



1: These bright layered deposits in the Valles Marineris region of Mars contain iron sulphates, which could support redox-driven microbial metabolisms. (NASA/JPL-Caltech/University of Arizona)

2: An SEM image of *Methanosarcina barkeri*, a methanogen (coccolidal cells), and *Geobacter metallireducens*, an iron-reducer (flattened rod-shaped cells). Both are obligately anaerobic (they are only found in environments lacking or devoid of oxygen) and are known to make a living by mediating redox reactions comprising minerals that have been identified on the martian surface. These microbes operate metabolisms that are plausible on Mars. (Sophie L Nixon)



into the martian atmosphere (Halevy *et al.* 2007) and the high-Fe composition of many martian basalts (McSween *et al.* 2009).

Sulphate terrains (e.g. figure 1) are distributed globally across Mars, comprising mono- and poly-hydrated Mg-sulphates, Ca-sulphates and (less commonly) Fe-sulphates (Bishop *et al.* 2009), within sedimentary (e.g. gypsum sands; Langevin *et al.* 2005) and hydrothermal (e.g. Gusev Crater; Ming *et al.* 2006) geological settings. Aside from the global nanophase ferric oxide/oxyhydroxide dust covering the martian surface (Banin *et al.* 1993), iron oxides and oxyhydroxides identified include haematite (e.g. Meridiani Planum; Klingelhofer *et al.* 2008), goethite (e.g. Gusev Crater; Morris *et al.* 2008), and other possible phases such as ferrihydrite and schwertmannite (Farrand *et al.* 2007). Haematite typically forms in standing bodies of liquid water, and is interpreted to be evaporitic in nature (Tosca *et al.* 2005), while this and other ferric oxide assemblages at Gusev Crater are associated with hydrothermal alteration of underlying basaltic crust (Morris *et al.* 2008). In many cases, sulphate and ferric oxide assemblages are associated with acidic hydrothermal processes (e.g. Juventae Chasma; Bishop *et al.* 2009, and Gusev Crater; Morris *et al.* 2008). The prevalence of S and Fe both globally, and in association with terrains thought to be formed or affected by liquid water activity, suggests Fe- and S-based metabolic strategies could have been exploited by martian life, if it was ever there. Additionally, there is significant overlap between Fe and S redox coupling, allowing for further metabolic pathways to be employed.

This article outlines iron and sulphur microbial metabolism and associated biogeochemical cycles within the context of martian environ-

ments inferred from hydrated mineral terrains. Methanogenesis is also discussed in light of the recent lack of detection within the martian atmosphere by the NASA Curiosity rover. Aside from understanding the potential for martian metabolism, this is also fundamental to the development of life-detection instrumentation. Metabolic processes significantly modify the immediate environment through redox cycling, mineral etching, gas release, isotope fractionation, and deposition of metabolic byproducts. These tell-tale signs of activity are specific to particular metabolic pathways, and are likely to constitute the main types of geochemical biosignature potentially identified within the martian rock record. Furthermore, if Mars is found to be devoid of life, these considerations provide an empirical basis from which to consider whether Mars is habitable, but devoid of life, or whether some factor, other than redox couples, may be absent, rendering martian environments uninhabitable.

Compatible microbial redox metabolisms

Many microorganisms can harness energy from reduction–oxidation (redox) reactions in the environment. These reactions involve a transfer of electrons from a donor to an acceptor molecule, and redox-driven microbes pass these electrons through electron transport chains, which ultimately establish a proton motive force used to synthesize ATP, the universal “energy currency” molecule (Madigan and Martinko 2006).

Numerous redox couples are known to support microbial metabolisms on Earth, including those involving iron (Fe), sulphur (S), nitrogen (N) and carbon (C), which can act either as

electron donors, acceptors, or both. For example, iron-oxidizing microorganisms mediate the oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron coupled to the reduction of oxygen or, in anoxic environments, nitrate (Straub *et al.* 1996). Other microorganisms can reduce the resulting ferric iron back to Fe(II) (e.g. *Geobacter metallireducens*; figure 2) using organic compounds, molecular hydrogen or elemental sulphur (among others) as electron donors (e.g. Caccavo *et al.* 1992, Coleman *et al.* 1993, Lovley *et al.* 1993). This represents the microbial iron cycle, one of many microbially mediated biogeochemical cycles.

Given the harsh surface conditions, lack of molecular oxygen, and mineral inventory known to date, compatible redox metabolisms on Mars centre on the cycling of Fe, S and methanogenesis. These are described in detail below.

Microbial oxidation of Fe and S

The martian crust is basaltic (McSween *et al.* 2009) and therefore dominated by iron-rich silicates such as olivine and pyroxene minerals (Boynton *et al.* 2008), with iron present predominantly as Fe^{2+} . As such, there is no shortage of Fe^{2+} as an electron donor for microbial iron oxidation on Mars. On Earth, most iron-oxidizing microbes utilize oxygen as a terminal electron acceptor, though many are capable of using nitrate (NO_3^-) in its absence (Straub *et al.* 2004), which is yet to be detected on Mars. It has been argued that perchlorate, detected by the Phoenix lander in martian soils (Hecht *et al.* 2009), could also be used as an electron acceptor (Coates and Achenbach 2004), though it is not clear whether this redox couple supports microbial growth as well as energy production. Because perchlorate originates in the



3: Fe- and S-rich terrestrial environments, such as these deposits in the volcanically active Krafla region of Iceland, serve as interesting analogue field sites for investigating redox-driven life. Studying the types of microorganisms present in these environments, and the hard limits of their metabolic processes, allows us to better define the habitability parameter space within which to search for signs of life on Mars. (Claire R Cousins)

atmosphere on Earth, it is thought to be globally widespread (Kounaves *et al.* 2010). Therefore, if further microbiology data demonstrates the use of perchlorate for microbial growth via iron oxidation, Mars can potentially host a full redox cycle for this metabolism (see table 1). Additionally, many iron-oxidizers can use CO₂ as the sole carbon source (e.g. Weber *et al.* 2006), a compound in abundance in the atmosphere of Mars, potentially providing a lithoautotrophic means of both energy generation and carbon assimilation.

Sulphide has not been detected at the martian surface by orbiting spacecraft, and only tentatively detected as a minor hydrothermal alteration product at Home Plate (Morris *et al.* 2008). However, igneous sulphide is likely to exist on Mars, and has been identified in martian meteorites (e.g. Burgess *et al.* 1989, Burns and Fisher 1990) suggesting its presence in the crust. Like iron, the majority of microbial sulphide oxidation occurs under aerobic conditions using O₂ as an electron acceptor, and under anoxia, NO₃⁻ can be utilized by some sulphide-oxidizing microbes (Kamp *et al.* 2006). With our current knowledge of martian geochemistry and microbiology, there is no known complete sulphide oxidation redox couple.

Microbial reduction of Fe and S

There are numerous sources of ferric iron and sulphate on Mars with the potential to support microbial iron and sulphate reduction. Among these, iron oxides and oxyhydroxides have long been identified as viable terminal electron acceptors for iron reduction. On Earth, nanophase

iron oxides such as ferrihydrite are the most widespread source of Fe(III) used in the natural environment (Lovley 2006), though more crystalline forms such as hematite and goethite are also used (Roden and Zachara 1996, Cutting *et al.* 2009). However, there are many more minerals bearing Fe(III) on Mars than have been tested as a source of electron acceptors; hence the actual range can likely be expanded. As with Fe(III), sulphates are widespread on Mars, both as a component within martian soil and within the near-surface crust. Sulphate and other oxidized forms of S, such as sulphite and thiosulphite, can be used to support sulphate-reduction, and represents one of the earliest bacterial metabolisms on Earth (Shen and Buick 2004).

Both Fe- and S-reducing microorganisms use a vast range of organic molecules (e.g. acetate or lactate) as an electron donor, which also serve as a source of carbon (e.g. Lovley and Lonergan 1990, Knoblauch *et al.* 1999). Indeed, many iron-reducing microbes are capable of reducing sulphates, and thus make use of much the same organic electron donors for metabolism. There is an apparent lack of these organic electron donors on Mars, with organics yet to be unambiguously identified *in situ* at the martian surface. The recent discovery, however, of magmatic reduced carbon within martian meteorites (Steele *et al.* 2012) highlights an endogenous source of carbon that, if released into the subsurface environment, could potentially be utilized by life. Additionally, the inventory of organic molecules to the martian

surface via meteoritic infall is estimated at 8.6×10^6 kg per year (Flynn 1996). Several of the organic molecules detected in carbonaceous meteorites are known electron donors (see Nixon *et al.* 2012 for a review). Therefore, if carbonaceous material survives delivery to the surface to become buried within the subsurface, a full redox couple exists to support microbial iron and sulphur reduction.

These metabolic pathways can also utilize molecular hydrogen (H₂) as an electron donor, and can use CO₂ as a carbon source (e.g. Kashefi *et al.* 2002). Hydrogen of photochemical origin is present in the martian atmosphere in trace quantities, and some argue that downward diffusion into the soil could render it an available energy source (Weiss *et al.* 2000). In addition, serpentinization could also support its production. This process involves the hydration of mafic minerals, and produces serpentine and other minerals, as well as dihydrogen (H₂) (Quesnel *et al.* 2009). With no shortage of the precursor minerals on Mars, and the detection of serpentine by CRISM (Ehlmann *et al.* 2010), it seems feasible that hydrogen was produced in the martian subsurface.

Finally, an alternative inorganic electron acceptor utilized by some iron reducers is carbon monoxide (CO) (e.g. Sokolova *et al.* 2004), another compound produced photochemically in the atmosphere and with the potential to be made available as an energy source through downward diffusion (Weiss *et al.* 2000). Furthermore, in the presence of water in highly reducing conditions some CO-utilizing microbes can derive all their energy and carbon through the oxidation of CO, without the need for additional electron acceptors such as Fe(III), producing H₂ and CO₂ as byproducts

“Several of the organic molecules detected in carbonaceous meteorites are known electron donors”

(Yoneda *et al.* 2012). Indeed, the presence of CO in the martian atmosphere in contact with surface Fe(III) minerals raises the question of whether this in itself demonstrates the lack of a biota on Mars (Weiss *et al.* 2000), although our knowledge of the sinks and sources of CO is still in its infancy.

Methanogenesis

Several lines of enquiry over the last few years have indicated the possible presence of localized and seasonally varying methane in the martian atmosphere (Krasnopolsky *et al.* 2004, Formisano *et al.* 2004, Mumma *et al.* 2009). These findings have been subject to debate (Zahnle *et al.* 2011) and recently the Mars Science Laboratory was unable to detect methane at the surface of Mars, at least at concentrations that would support the hypothesis of a biological source, although potential martian sinks of subsurface methane are not fully known. Most methanogens on Earth acquire energy from the reduction of CO₂ using molecular hydrogen (e.g. *Methanosarcina barkeri*; figure 2), and the aforementioned production of hydrogen through serpentinization is commonly cited as a driver of possible methanogenesis in the subsurface (e.g. Parnell *et al.* 2010). Some methanogens produce methane through the oxidation of CO in the presence of water (Daniels *et al.* 1977).

Knowledge gap

With the exception of a few martian meteorites, our knowledge of martian geochemistry is limited to the surface. It is generally agreed that the subsurface of Mars is where the search for life should be focused, given the inhospitable nature of the subaerial environment. Liquid water, sources of energy, and organics could render subsurface regions of Mars habitable today, though until we are able to drill beneath the surface it is difficult to assess the localization and concentration of plausible redox couples, or other physical factors and chemical elements required for life.

Our understanding of the feasibility of potential redox couples on Mars is currently very restricted, simply because very few martian minerals and relatively few meteoritic organics have ever been tested as a means of metabolism. For instance, it is unknown whether such minerals as nontronite or jarosite represent viable sources of electron acceptors, despite bearing ferric iron. It is also unknown whether an Fe- or S-reducing microbe could access and use organics from carbonaceous meteorites, where the fraction containing known electron donors represents no more than 2% in most specimens

Table 1: Potential metabolic species thought to be present on Mars

electron donors	electron acceptors
Fe ²⁺ : available in Fe-rich silicates	Fe ³⁺ : available in numerous alteration minerals
H ₂ : available in subsurface?	SO ₄ ²⁻ : available in salts
CO: available in atmosphere	O ₂ : partial pressure too low
organics: meteoritic likely to be present at surface	NO ₃ : presence or abundance unknown
organics: endogenous available in subsurface	ClO ₄ : available but not shown to support growth

(Sephton 2002). Similarly, the lack of knowledge on whether serpentinization could drive biologically available H₂ production on Mars limits our ability to assess hydrogen as an electron donor.

“Liquid water, sources of energy, and organics could render subsurface regions of Mars habitable today”

In general, Mars appears to be rich in electron acceptors for S- and Fe-reduction (sulphates, ferric minerals), but lacking in donors (organic carbon, H₂). In contrast, electron donors for S- and Fe-oxidation (sulphides, ferrous minerals) exist, but electron acceptors such as nitrates are possibly lacking or highly localized. While there has been significant work exploring the microbiology of terrestrial environments analogous to acidic, Fe- and S-dominated process on Mars (e.g. Sánchez-Andrea *et al.* 2011; figure 3), there is a need now to fine-tune these microbiological models to apply more directly to martian geochemistry. Additionally, greater efforts to understand the potential for a more diverse range of anaerobic redox couples to drive energy acquisition is required, along with their corresponding distribution on Mars. ●

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